IN THE SPECIFICATION:

Applicants, pursuant to 37 C.F.R. § 1.121, submit the following amendments to the specification:

Kindly ***substitute*** the following contiguous paragraphs for the corresponding paragraphs beginning on page 43 and extending through page 47 of the originally filed specification:

--EXAMPLE 1

Organ specific methylation pattern analysis on plasma samples

A blood sample is~~was~~ taken from a patient who is~~was~~ unaware that he had been exposed to high levels of radiation during his years of service at the army. Now he wishes to know whether he has developed a neoplastic disease like a tumour. His physician has not yet found any typical symptoms other than the patient complaining about unspecific pain at different organs, including headache.

A 20 ml blood sample is~~was~~ collected in heparin. Plasma and lymphocytes are~~were~~ separated by Ficoll gradient. Control lymphocyte and plasma DNA are~~were~~ purified on Qiagen columns (Qiamp Blood Kit, Qiagen, Basel, Switzerland) according to the "blood and body fluid protocol". Plasma is~~was~~ passed on the same column. After purification of about 10 ml of plasma 350 ng of DNA are~~were~~ obtained. The DNA is~~was~~ subjected to a sodium bisulfite treatment as it has been described in Olek A, Oswald J, Walter J. (1996) A modified and improved method for bisulphite based cytosine methylation analysis. Nucleic Acids Res. 24: 5064-6. An aliquot of this bisulfite treated DNA is~~was~~ used for methylation analysis based on sequencing. The individual's test result is~~was~~ compared with the dataset obtained from previous samples of known tissues and cell types as it is shown in figure 7. From that it can~~could~~ be concluded that a significant portion of the DNA in the patient's blood is~~was~~ derived from his lung. Said result is sent~~was send~~ back to the physician who now refers~~referred~~ the patient to a hospital that is specialised on inflammatory or

cell proliferative diseases of the lung.

EXAMPLE 2

Organ specific methylation pattern analysis on serum samples

A blood sample is~~was~~ taken from a patient, who wishes to know whether he has developed a neoplastic disease like a tumour. His physician has not yet found any typical symptoms other than the patient complaining about randomly occurring unspecific pain in his stomach, recurrent headache and pain in his kidneys.

A serum sample is~~has been~~ taken from the patient. DNA is~~has been~~ isolated from the serum with the use of the Qiamp kit and is~~has been~~ bisulfite treated as described in Example1.

A typical methylation pattern can~~could~~ be determined analysing the methylation statuses of a higher number of different informative CpG sites, than is~~that were~~ used as markers for different tissues and organs, simultaneously. That is~~was~~ done by first amplifying the relevant fragments with the use of specific primers designed as to only specifically amplify those fragments of the bisulfite treated DNA that contain informative CpG positions. These amplificates are~~were~~ labelled with a fluorescent dye. A set of detection oligos, each designed as to specifically only hybridise with the amplified version of the bisulfite treated nucleic acid that is~~was~~ methylated as it is characteristic for a specific organ. The detection oligos contain a CG when said CpG position is methylated in a specific organ or tissue (or a TG where said CpG position is unmethylated in a specific organ or tissue). These oligos are~~were~~ fixed to a solid surface as to provide a chip. The labelled amplificates are~~were~~ hybridised with said chip and non hybridising amplificates are~~were~~ removed. The signal pattern on the chip is~~was~~ then translated in a methylation pattern, indicative of a specific organ.

The analysis of the patient's DNA methylation patterns, leads~~led~~ to the conclusion that a significant portion of the DNA originated from colon.

The physician therefore initiates~~initiated~~ a second analysis on said bisulfite treated DNA. He requires~~required~~ the patient's DNA to be tested a second time, this time specifically only with the colon marker EYA 4. A predominant signal can~~could~~ be detected using the following EYA4-HeavyMethyl MethyLight assay. The methylation status is~~was~~ determined with a HM MethyLight assay designed for the CpG island of the EYA4 colon marker gene and a control gene was assayed in parallel. The CpG island assay covers CpG sites in both the blocking oligos and the taqman® style probe, while the control gene does not.

Methods. The CpG island assay (methylation assay) is~~was~~ performed using the following primers and probes:

Control gene : beta actin (Eads et al., 2001):
Primer: TGGTGATGGAGGAGGTTTAGTAAGT (SEQ ID No. 1);
Primer: AACCAATAAAACCTACTCCTCCCTTAA (SEQ ID No. 2); and
Probe: ACCACCACCCAACACACAATAACAAACCA (SEQ ID No. 3)

EYA4 gene
Forward Primer: GGTGATTGTTTATTGTTATGGTTTG (SEQ ID No. 4)
Reverse Primer: CCCCTCAACCTAAAAACTACAAC (SEQ ID No. 5)
Forward Blocker: GTTATGGTTTGTGATTTTGTGTGGG (SEQ ID No. 6)
Reverse Blocker: AAACTACAACCACTCAAATCAACCCA (SEQ ID No. 7)
Probe: AAAATTACGACGACGCCACCCGAAA (SEQ ID No. 8).

The reactions are~~were~~ each run in triplicate on the individual's DNA sample with the following assay conditions:

Reaction solution: (400 nM primers; 400 nM probe; 10μM both blockers; 3.5 mM magnesium

chloride; 1x ABI Taqman buffer; 1 unit of ABI TaqGold polymerase; 200μM dNTPs; and 7μl of a solution containing 50 ng of DNA, in a final reaction volume of 20 μl);

Cycling conditions: (95°C for 10 minutes); (95°C for 15 seconds, 64°C for 1 minute (2 cycles)); (95°C for 15 seconds, 62°C for 1 minute (2 cycles); (95°C for 15 seconds, 60°C for 1 minute (2 cycles)); and (95°C for 15 seconds, 58°C for 1 minute, 60°C for 40 seconds (41 cycles)).

The amplification of said fragment indicates~~indicated~~ the presence of a specific methylation pattern in said informative CpG positions (of EYA 4). From comparing the test result and the intensity of the fluorescent signal with a data set obtained from other samples it can~~could~~ be concluded that a significant part of the DNA in the patient's~~patients~~ sample originated from colon. This result allows~~allowed~~ the physician to refer said patient to an expert in gastrointestinal diseases.

EXAMPLE 3

In another case the physician follows~~was following~~ a different strategy. He is~~was~~ first testing for the total amount of free floating DNA in said patient's serum, because this test is less cost intense and is~~was~~ covered by the patient's insurance. The blood sample is~~was~~ sent to a laboratory. After separating~~having separated~~ the plasma from blood cells by centrifugation at 3000g for 20 min the DNA from the blood plasma is~~was~~ extracted using the QIAamp Blood Kit (Qiagen, Hilden, Germany) using the blood and body fluid protocol referring to Wong et al. (1999), Cancer Res 59: 71-73 and Lo et al. (1998) Am. J. Genet. 62: 768-775. It is~~was~~ determined that the level of total free floating nucleic acids in said serum sample is~~was~~ 20 times higher than it usually is in samples from healthy donors, that are not suffering from cell proliferative diseases. The data ~~that were~~ establishing this "normal" value are~~have been~~ obtained from~~in~~ previous studies based on a high number of samples and are~~were~~ approved by the regulatory agencies. These data being~~had been~~ stored in their dataset.

Knowing that his patient hashad a level of free floating DNA in his serum that iswas 20 times higher than the average allowsallowed the physician to diagnose a high likelihood of his patient to suffer from a cell proliferative disease. With this diagnosis the insurance iswas willing to pay for a more informative test as to further specify the kind of disease.

The physician now requestsrequested the methylation analysis of said DNA with the aim to determine where the free floating DNA in the serum of his patient originated from. Said DNA iswas treated with sodium bisulfite as described above. The methylation pattern analysis iswas carried out with the use of a number of informative CpG site containing marker nucleic acids and the collected datasets from other samples to compare the results with (as illustrated in figure 4). Said analysis revealsrevealed that a significant portion of said free floating DNA originatesoriginated from liver. At this point the physician refersreferred the patient to an oncologist specified for liver tumours.

EXAMPLE 4

A research team is interested in identifying risks of developing lung specific diseases like for example lung cancer in a population, that has been exposed to specific environmental conditions. As these conditions only developed during the recent years, no data are available on an accumulated occurrence of cancer in said population yet. Therefore the team isthey are employing an analysis of said individual'sindividuals bodily fluids as to whether they can find early signs of developing diseases. Sputum samples arehave been collected from a high number of individuals.

Those sputum samples arewere analysed as follows: Sputum samples arewere spun at 3000 x g for 5 min and washed twice with phosphate-buffered saline. Cell pellets arewere digested with 1% SDS/proteinase K, and DNA iswas extracted and purified using Qiagen columns (Qiamp Blood Kit, Qiagen, Basel, Switzerland) according to the "blood and body fluid protocol". The DNA obtained iswas subjected to a sodium bisulfite treatment as it has been described in Olek A, Oswald J, Walter J. (1996) A modified and improved method for bisulphite based cytosine

methylation analysis. Nucleic Acids Res. 24: 5064-6. An aliquot of this bisulfite treated DNA is~~was~~ used for methylation analysis. As the study is~~was~~ designed to only look for lung diseases, the analysis is~~was~~ restricted to the use of informative CpG sites that are specifically methylated in lung cells, but unmethylated in other cells that might potentially occur in a sputum sample. The methylation analysis is~~was~~ based on sensitive detection assays. First results are~~were~~ obtained with the use of a HM assay, as it is described in here. Primers are~~were~~ designed to amplify a fragment that contains seven different CpG sites that are all methylated only in DNA from lung cells. Blocking oligos are~~were~~ designed that hybridised to two of those sites in the bisulfite treated DNA, only when said CpG sites are~~were~~ unmethylated prior to bisulfite treatment. A pair of Lightcycler probes is~~was~~ designed as to only bind to the amplified fragment of the bisulfite treated DNA when two different informative CpG sites are~~were~~ methylated. That way, the presence is~~was~~ indicated by the generation of a fluorescent signal and the amount of said lung derived DNA in the total amount of DNA is~~was~~ quantified by the number of cycles required to generate a detectable signal in comparison to signals generated by standardised amounts of control DNA.

The primary test results are~~have been~~ confirmed with the use of MSP primers in combination with the use of Taqman probes. MSP primers are~~were~~ designed to specifically bind to the bisulfite treated sequence containing two and three of those CpG sites that are~~were~~ methylated in lung cells, but not in other cells. The Taqman probe is~~was~~ designed to bind to the other two CpG sites in said amplified product only when those are~~were~~ unmodified after treatment with bisulfite (methylated cytosines prior to treatment). Therefore the presence of an amplification product, indicated by the fluorescent signal of the Taqman probe confirms~~confirmed~~ the primary results.

As the majority of the individuals do~~did~~ not show free floating DNA in their sputum samples that exhibits~~exhibited~~ methylation pattern characteristic for lung, it is~~was~~ concluded that they do~~did~~ not contain lung derived DNA in their sputum samples. It is~~was~~ concluded that said population did not develop lung specific cell proliferative diseases and as such there s~~was~~ no reason to believe that said environmental conditions are~~were~~ adding to the risk of developing a neoplastic or

inflammatory diseases like lung cancer or lung inflammation.